Sveriges lantbruksuniversitet
Fakulteten för Veterinärmedicin och husdjursvetenskap
Institutionen för Biomedicin och veterinär folkhälsovetenskap

Tick-borne diseases in Tajikistan
- anaplasmosis, babesiosis and theileriosis

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SAMMANFATTNING

Tajikistan ligger i Centralasien och är den fattigaste av 15 före detta sovjetrepubliker. Cirka 60% av befolkningen lever i fattigdom. Mer än 70% av hushållen äger boskap, och friska djur är därför viktigt för att bekämpa fattigdom i landet.

Fästingburna sjukdomar orsakar stora direkta och indirekta inkomstbortfall och är ett stort hinder för tillväxt inom jordbrukssektorn framförallt i områden där man är beroende av boskap. På grund av svårigheter att beräkna exakta inkomstbortfall är detta ofta ett negligerat problem. Direkta effekter av fästingangrepp är nedsatt värde på hudar som fått märken efter fästingbett. Indirekta effekter är sjukdomar som sprids med fästingar och orsakar nedsatt produktion av kött och mjölk, samt sjukdom och död hos djuren som drabbas.

Anaplasmos, babesios och theilerios är sjukdomar som sprids med fästingar och förekommer i stora delar av världen, däribland Centralasien. Få studier är gjorda om dessa sjukdomars utbredning och skadeverkan i detta område.

Målet med min studie var att undersöka förekomsten av *Anaplasma marginale* och *Babaeisa bigemina*, som orsakar anaplasmos och babesios, hos mjölkkor i västra Tajikistan samt att identifiera riskfaktorer relevanta för sjukdomskontroll.

Blodprover samlades in från 294 mjölkkor på sju statliga enheter och tre privata. Blodproverna analyserades med avseende på förekomst av antikroppar mot *A. marginale* och *B. bigemina*. Jag undersökte också om det förelåg någon skillnad i seroprevalens mellan grupper avseende ålder, ras, statlig/privat enhet, region, antal fästingar på djuren och om de gick på bete eller inte. Den totala seroprevalensen bland de provtagna djuren var för *A. marginale* 14% och för *B. bigemina* 18%. Inga faktorer som påverkar förekomsten kunde identifieras med statistisk säkerhet, men mellan statliga och privata enheter observerades en numerär skillnad i seroprevalens. Seroprevalensen för både *A. marginale* och *B. bigemina* var högre bland de provtagna djuren på privata enheter jämfört med statliga. Orsaken till denna numerära skillnad är oklar, men skulle kunna bero på att man håller djuren på olika sätt till exempel med avseende på betes- och fästingprofylaxrutiner på statliga enheter jämfört med privata enheter. För att fastställa orsaker till skillnaderna skulle det behövas ytterligare studier i ämnet där man undersöker enskilda yttre faktorer var för sig.

SUMMARY

Tajikistan is situated in central Asia and is the poorest of 15 former Soviet republics. Sixty percent of the people in the country live below poverty line. Over seventy percent of the households in Tajikistan own cattle, and healthy animals are therefore very important to reduce poverty in the country.

Tick-borne diseases (TBDs) directly and indirectly cause a significant reduction in income and hamper the growth of the whole agricultural sector, which is especially serious in livestock dependent systems. Due to difficulties in estimating income losses caused by TBDs these problems are often neglected. The direct effect of tick infestation is reduction of the value of hides because of tick marks. Indirect effects are diseases transmitted by ticks that cause losses in production of milk and meat, and illness and deaths among affected cattle.

Anaplasmosis, babesiosis and theileriosis are diseases in cattle transmitted by ticks in large parts of the world, including Central Asia. Few studies have been made on the spread of and damage caused by these diseases in the area.

The aim of this study was to investigate occurrence of *Anaplasma marginale* and *Babesia bigemina*, which cause anaplasmosis and babesiosis, in dairy cattle in western Tajikistan and to study risk factors relevant for controlling these diseases.

Blood samples were collected from 294 cattle at seven governmental units and three private units. The samples were analyzed for presence of antibodies against *A. marginale* och *B. bigemina.* Divergence in seroprevalence between groups considering age, breed, governmental/private units, region, number of ticks and pasture/no pasture were also investigated. The overall seroprevalence among the sampled animals was 14% for *A. marginale* and 18% for *B. bigemina*. No statistically significant risk factors affecting seroprevalence were identified, but there was a clear numerical difference in seroprevalence between governmental and private units. The seroprevalence was higher among cattle sampled at private units compared to governmental. The cause of this numerical difference is unclear, but could be due to different grazing- and prophylaxis routines at governmental and private units. To appoint the cause of this difference in seroprevalence further studies are needed where possible risk factors are investigated one at a time.

INTRODUCTION

Tajikistan

Tajikistan is situated in Central Asia and borders in the north to Kyrgyzstan, in the west to Uzbekistan, in the east to China and in the south to Afghanistan (map 1). The total area of the country is 143.100 sqkm and the landscape is dominated by the Pamir mountains, with its highest point Qullai Ismoili Somoni 7.495 meters above sea level, in the east and Alay mountains in the north (CIA, 2008). More than 85% of the land is found at altitudes between 1500 and 1800 meters above sea level (Magnusson et al., 2005). The two major lowland areas are western Fergana Valley in the north and Kofarnihon and Vakhsh Valleys in the southwest (CIA, 2008).

Map 1. Map over Tajikistan (https://www.cia.gov/library/publications/the-world-factbook/geos/ti.html).

The climate varies greatly depending on altitude. It is midlatitude continental with hot summers and mild winters in the lowland areas, whereas it is semiarid to polar in the mountainous areas like the Pamirs (climate-zone, 2008).

The country is a former Soviet Union republic and became independent in 1991. In 1992-97 there was a civil war that severely damaged the already weak economic situation and caused a marked decline in industrial and agricultural production (Wikipedia, 2008). Now Tajikistan is the poorest of the 15 republics of the former Soviet Union with a GDP-per capita of $1.600 (CIA, 2008).

Tajikistan is populated by 7.2 million people. The main ethnical group is Tajiks (79.9%), followed by Uzbeks (15.3%), Russians (1.1%) and Kyrgyz (1.1%). Of the people, 85% are Sunni Muslims and 5% are Shia Muslims. The official language is Tajik, which is a variety of Persian, but Russian is largely spoken in business and for government purposes (CIA, 2008).
Life expectancy at birth in Tajikistan is 66 years and the under-five mortality rate is 7%. As many as 43% of the population, lives below $2 a day (UNDP, 2008).

Livestock

In Tajikistan 70% of the people live in rural areas and about 70% of the rural households own livestock. Also many households in urban areas own livestock. Since so many people are dependent on livestock as a source of meat and milk it is very important that animals are healthy and productive to reduce poverty in the country (Magnusson et al., 2005).

In the villages the animals are grazed collectively as a unit. Many farmers take their animals to high altitude summer pastures where animals from several villages graze together. Owing to poor utilization of pastures, uncontrolled breeding, low reproduction rates and diseases including internal and external parasites the productivity of the livestock is generally low (Jackson et al., 2007). The livestock production is mostly based on grazing. The increasing number of cattle has resulted in problems with overgrazing and erosion and in wintertime there is often a feed shortage that leads to metabolic diseases.

In 2003 the approximate number of cattle in Tajikistan was 1.140.000. More than 85% of the cattle were owned by private small holders like the ones mentioned above. Approximately 120.000 (10%) were held in state farms and 30.000 (2,5%) were held in dehkans which are former state and collective farms. State farms and dehkans hold a greater number of animals for milk production. The milk production per cow in Tajikistan is estimated to about 2000 kg/year.

The governmental support to the animal health services has been much reduced since independence, and today there is poor access of veterinary medicines and vaccines, inadequate control of infectious diseases and difficulties in diagnosing and reporting diseases. The livestock health is in general poor and the prevalence of infectious diseases is high. The zoonotic diseases anthrax, brucellosis, tuberculosis, rabies and echinococcus are considered as public health threats in Tajikistan by the national authorities (Magnusson et al., 2005).

Tick-borne disease

Animal diseases including tick-borne diseases (TBDs) are one factor that seriously restrain, both directly and indirectly, the growth of livestock and the whole agricultural sector (de Castro, 1997). Especially livestock-dependent systems are vulnerable to the reduction in income that TBDs cause (Ilham Rasulov., 2007). Based on Australian figures, assuming that 80% of all the cattle in the world are at risk of getting infected with TBDs, the global losses from these diseases in cattle are estimated to be between USD 13.9 and 18.7 billion per annum (de Castro, 1997). The direct effect of tick infestation is reduction of the value of hides because of tick marks. Indirect effects are transmission of diseases that causes reduction in milk and meat production, illness and deaths (Ilham Rasulov, 2007).

Babesia and *Theileria* infections are presumably the most widespread TBDs and livestock worldwide are affected by pathogenic species of these parasites (Bakheit et al., 2007).

Anaplasma infection is also one of the most important TBDs (Rajput et al., 2005), causing infections in cattle in large parts of the world, including Central Asia (Razmi, 2006).

TBDs are divided in four major complexes affecting cattle around the world. Two of these complexes affect cattle in Asia (de Castro, 1997):

I. Ticks belonging to *Boophilus spp* (picture 1) and the pathogens *Babesia spp* (babesiosis) and *Anaplasma marginale* (anaplasmosis).

This complex occurs in Latin America, Oceania, Asia, large areas of Africa and the Near East. It is presumed to be the most important complex and the ticks cause direct and indirect losses which particularly affect imported and crossbred high producing dairy cattle (de Castro, 1997).

II. Ticks belonging to *Hyalomma spp* (picture 2) and the pathogen *Theileria annulata* (tropical theileriosis).

This complex is mainly distributed across northern Africa, southern Europe, the Near East and West Asia. In Asia the systems most affected by this complex are small holders, periurban dairies and imported cattle (de Castro, 1997).

Picture 1. Adult stage of Boophilus tick (http://student.britannica.com/elementary/art-7772 (20090415))

Picture 2. Picture of a Hyalomma marginatum tick (//en.wikipedia.org/)

Anaplasmosis

Etiology

Anaplasmosis in cattle and wild ruminants is caused by infection of *Anaplasma marginale* or *A. centrale*. *Anaplasma spp.* are obligate intraerythrocytic parasites belonging to the order Rickettsiales (Radostits et al., 2007).

Epidemiology

Anaplasmosis in cattle is distributed worldwide being present in Africa, southern Europe, Australia, Asia, the former USSR, South America and the United States (Taylor et al., 2007). The prevalence of infection in cattle in endemic areas is often very high with seropositivity rates between 60% and 90%. Seropositivity is much lower in regions that interface between endemic and non-endemic regions (Radostits et al., 2007). Clinical disease is rare in endemic areas and symtoms mainly occur when susceptible animals are introduced into endemic areas with mortality rates sometime reaching 80% (Taylor et al., 2007). Clinical disease also occurs where there is expansion of the vector population into previously free areas or in the interface between endemic and non-endemic regions (Radostits et al., 2007).

Transmission

The source of infection with *A. marginale* is the blood of an infected animal. Important natural vectors are ticks in the family Ixodidae. The one-host *Boophilus spp.* are of major importance in tropical and subtropical regions. Except from transmission via ticks the agent can also be transmitted transplacentally, mechanically by biting flies or iatrogenically (Radostits et al., 2007).

Anaplasma marginale has also been transmitted experimentally with a number of species of *Tabanus* (horseflies), and with mosquitoes of the genus *Psorophora*. It is unclear how big the importance of biting insects is in the natural transmission of anaplasmosis (OIE, 2008).

Susceptibility

Bos indicus, *Bos taurus* and their crosses have equal susceptibility to infection and show the same age susceptibility, but under field conditions *Bos indicus* are not as commonly affected, probably because of their relative resistance to heavy tick infestation (Radostits et al., 2007). Age at infection is a major determinant of the severity of clinical disease. Young calves are less susceptible to infection with *A. marginale* than older cattle (Taylor et al., 2007), and when infected, are less susceptible to clinical disease (Radostits et al., 2007). Animals infected between two and three years of age have increased risk of clinical disease and infection in animals older than three years of age often result in a peracute fatal form of the disease (Taylor et al., 2007). Clinical disease is less severe in cattle with poor nutrition status (Radostits et al., 2007).

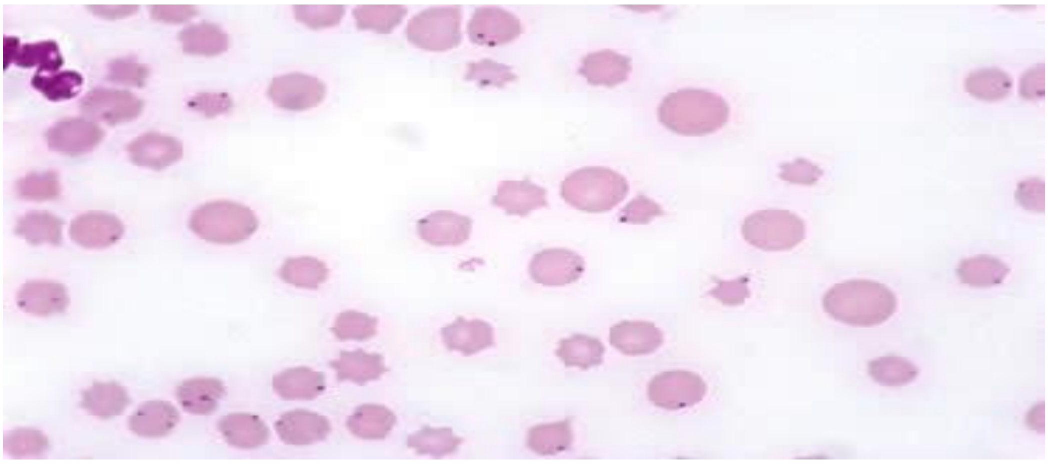
Pathogenesis and clinical signs

The incubation period is about 3-4 weeks. From 10-90% of all mature erythrocytes may be parasitized in the acute stage of the infection. At least 15% have to be parasitized to cause clinical disease. Erythrocyte destruction results in the development of mild to severe anaemia and icterus (Radostits et al 2007). There is no haemoglobinaemia and haemoglobinuria which is a way to differentiate between anaplasmosis and babesiosis, which often are endemic in the same areas (OIE, 2008). Fever and loss of appetite are other symptoms of the disease. Death can occur, but most animals survive in an emaciated condition with impaired fertility. Animals that survive the initial attack become persistently infected and this is a state characterized by intermittent rickettsemia. These cattle become carriers and are sources of infection. The symptoms in peracute cases are high fever, anaemia, icterus, severe dyspnea and death. The animals may become hyperexcitable and pregnant cows frequently abort. Bulls may get depressed testicular function (Radostits et al., 2007).

Diagnosis, treatment and prophylaxis

The traditional method of identifying *Anaplasma* in clinically affected animals is to examine Giemsa stained blood or organ smears (picture 3). In these smears *A. marginale* are seen as dense, rounded, intraerythrocytic bodies approximately 0.3-1.0 µm in diameter situated on or near the margin of the erythrocyte (OIE, 2008). Reduced erythrocyte count and immature red blood cells are also signs of the disease. Other diagnostic methods are indirect fluorescent antibody (IFA) test and indirect or competitive inhibition ELISA tests (Radostits et al., 2007).

The complement fixation (CF) test is the standard test to detect carrier animals. Previously transmission to spleenectomized animals was used to detect carriers, but since this method is expensive it is now replaced by PCR where this technique is available.



Picture 3. Anaplasma marginale infection in bovine blood, Wright-Giemsa, 100X oil immersion (www.merchvetmanual.com)

Clinical anaplasmosis can be treated with tetracyclines or imidocarb, but the convalecence period is long. Tetracycline treatment does not eliminate infection and the treated animal will get persisting immunity. Administration of estradiol cypionate under ongoing treatment with tetracyclines improve numbers of recovered animals by promoting parasitemia during treatment. If the animal is very sick with low packed cell volyme, blood transfusion is necessary.

The disease is controlled for by controlling arthropods with ascaricides, chemotherapy for prevention and vaccination. Carriers should not be introduced into herds and iatrogenic transmission should be avoided. Naive cattle introduced to endemic areas should be vaccinated and moved there when less than two years old and at a time when there are low numbers of insects (Radostits et al., 2007).

Vaccines

There are *A. centrale*-based vaccines that also protect against *A. marginale*, and there are combined vaccines against *B. bigemina*, *B. bovis* and *A. marginale* (de Castro, 1997). The *A. centrale*-based vaccines which provides partial cross-protection against *A. marginale,* are the most accepted. Strains of *A. marginale*, attenuated by passage in nonbovine hosts, are sometimes used for vaccination (OIE, 2008).

Babesiosis

Etiology

Babesiosis in cattle is caused by intra-erythrocytic protozoan parasites of the genus *Babesia*, order Piroplasmida, phylum Apicomplexa (Bakheit et al., 2007).

Epidemiology

Babesia spp. are one of the most wide spread blood parasites in the world (Bakheit et al., 2007). *Babesia bovis* and *B. bigemina* affects cattle and are of major economical importance in cattle industry in Africa, Asia, Australia, and Central and South America (OIE, 2008). *Babesia bigemina* and *B. bovis* are spread in the same areas where *Boophilus* ticks are present (Bakheit et al., 2007).

Transmission

Transmission of *Babesia spp.* is transovarial by *Ixodid* ticks of *Boophilus* species (Bakheit et al., 2007).

Susceptibility

Bos indicus breeds are more resistant to babesiosis than *Bos taurus* breeds. There is age related immunity to primary infection of cattle with *B. bigemina* and the severity of clinical signs increases with age. Susceptibility decreases with age (Radostits et al., 2007).

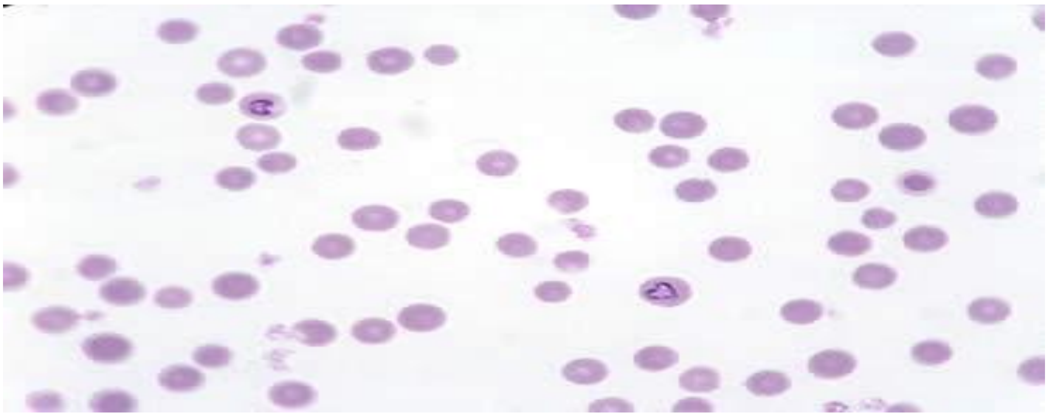
Pathogenesis and clinical signs

Parasites are inoculated in the blood stream by ticks. The incubation period is 7-20 days and the clinical signs that last up to 7 days are high fever, inappetence, ruminal atony, depression, weakness, roughened hair coat, anaemia, dyspnoea, tachycardia and hemoglobinuria. Affected animals recover slowly and there is a severe weight loss and drop in milk production. Pregnant cattle may abort. Mortality rates are variable and sometimes reaches 50% (Taylor et al., 2007). Protozoa survive in the host approximately six months after infection and if not reinfected immunity lasts at least four years. An animal that is reinfected several times get permanent immunity to the disease. Infected animals may become carriers when protozoas remain in the blood stream causing a mild subclinical infection. If the carrier animal is stressed it may develop clinical signs (Radostits et al., 2007).

There seem to be some degree of cross-protection in *B. bigemina*-immune animals against *B. bovis* infections. Calves usually show no clinical signs after infection, regardless of the *Babesia* spp. involved, and the immune status of the dams does not influence the calves resistance (OIE, 2008).

Diagnosis, treatment and prophylaxis

It is possible to find the protozoa in Giemsa-stained capillary blood smears (picture 4). CF-, IFA- and ELISA tests are serological tests to diagnose past or present infection. The parasite may also be detected by PCR technique. Carrier cattle are hard to detect (Radostits et al., 2007).



Picture 4. Babesia bigemina parasites in two erythrocytes, bovine blood, Wright-Giemsa, 100X oil immersion (www.merchvetmanual.com)

Formerly quinuronium sulfate, amicarbalide isoethionate and diminazene aceturate were used for treatment. Now the drug of choice for treatment of babesiosis is imidocarb. Control is dependent on tick control, by acaricides, chemoterapy in infected animals and immunization (Radostits et al., 2007).

Vaccines

There are live attenuated vaccines to prevent infection with *B. bovis* and *B. bigemina*, produced from the blood of infected animals. It is common with vaccine reactions and therefore it is not recommended to vaccinate animals older than one year. It takes 3–4 weeks to develop protective immunity that lasts for several years, after a single vaccination (OIE, 2008).

Tropical theileriosis

Etiology

Tropical theileriosis is caused by *Theileria annulata* wich is an apicomplex protozoan parasite. It is transmitted by the *Hyalomma* ticks (Radostits et al 2007). These parasites are obligate intracellular agents that infect both wild and domestic *Bovidae*. Their life cycle is complex, and takes place in both vertebrate and invertebrate hosts (OIE, 2008).

Epidemiology

The disease is a major constraint to livestock improvement programmes in many parts of the Middle East and Asia. It occurs from Western Europe through the Middle East, to China. In endemic areas almost all adult animals are infected and the case-fatality rate is around 10-20%. When exotic animals are introduced the mortality may range between 20 and 90% (Radostits et al., 2007).

Transmission

In central Asia *T. annulata* is transmitted by ticks of the genus *Hyalomma*. Buffaloes are often carriers and are believed to be a natural reservoir.

Susceptibility

Cattle of local breeds are often subclinically infected by *T. annulata* while European dairy cattle are more susceptible and show clinical signs. Exotic animals introduced to an endemic area are at high risk (Taylor et al., 2008). Clinical signs in cross breeds are milder than in exotic breeds. In endemic areas case-fatality is 10-20% and it is mostly calves that get diseased (Radostits et al., 2007).

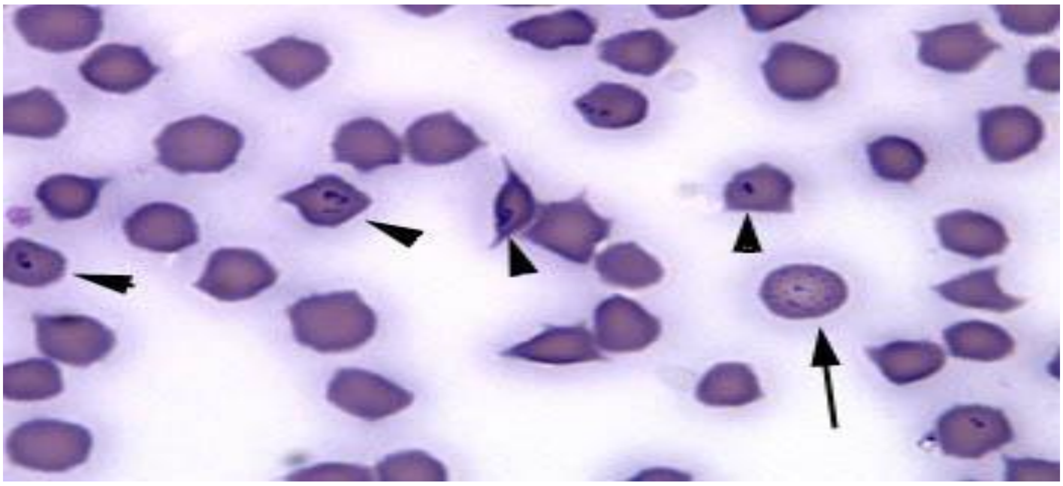
Pathogenesis and clinical signs

The life cycle of *T. annulata* is cattle-tick-cattle. The parasite infects and forms schizonts mostly in macrophages and monocytes. Schizonts spread through draining lymph nodes and disseminates throughout lymphatic and non lymphatic tissues. The pathogenesis involves proliferation of lymphocytes and macrophages induced by proliferating schizonts and anemia with icterus induced by piroplasms which invade red blood cells (Radostits et al., 2007).

Clinical findings are fever, swelling of superficial lymph nodes, inappetence, tachycardia, dyspnea, pale mucus membranes and icterus. In chronic cases there are sometimes small subcutaneous nodules. Animals that recover get a long lasting immunity, but become carriers (Radostits et al., 2007).

Diagnosis, treatment and control

Diagnosis is often based on clinical signs together with knowledge of presence of the disease, vector distribution and parasite detection in smears of blood and lymph tissue. Giemsa-stained blood and lymph node smears reveal piroplasms in erythrocytes and schizonts in lymphocytes of acute infected animals (OIE, 2008)(picture 5).
Serology tests available are indirect flouorescence antibody test and ELISA. Carriers can be detected by PCR (Radostits et al., 2007).



Picture 5. Theileria piroplasms in bovine red bloodcells (www.vet.uga.edu/VPP/clerk/siegel/fig03.jpg)

The most effective agent to treat tropical theileriosis is buparvaquone, a hydroxynaphthoquinone antiprotozoal drug related to parvaquone and atovaquone. There is also halofuginone and tetracyclins which is not considered as effective as buparvaquone (Radostits et al., 2007). To prevent disease it is important with tick control (OIE, 2008).

Vaccines

There is a schizont-based *T. annulata* vaccine that is widely used (de Castro, 1997). The vaccine is prepared from cell lines infected with schizonts that have been isolated from infected cattle and attenuated during in-vitro culture (OIE, 2008).

INTRODUCTION TO STUDY

This study was conducted in Tajikistan during the month of September 2008 in cooperation with the Tajik Agrarian University (TAU) and the Food and Agricultural Organisation (FAO) in Dushanbe, Tajikistan. The field study was conducted together with my friend Elisabeth Lindahl, veterinary student at the Swedish University of Agricultural Sciences in Uppsala, Sweden.

As far as I know, there has been no previous seroprevalence study on *T. annulata* and *B. bigemina* on dairy cattle in Tajikistan. A study of 3 285 bloodsamples and punctata of lymphatic nodes from diseased and emergency slaughtered cattle in Uzbekistan showed that 56% of cattle in the study area were infected by *T. annulata* and 23% of the cattle by *B. bigemina* (Ilham Rasulov, 2007).

One study made in Tajikistan showed that among 426 cattle from 15 farms in 5 different regions the seroprevalence for *A. marginale* was 18% (Sakhimov, 1997).

The diseases to be included in this study were selected in cooperation with FAO and TAU. Because of the absence of a diagnostic method suitable for the conditions prevailing within this project for testing occurrence of antibodies against *T. annulata* this pathogen was excluded from the study.

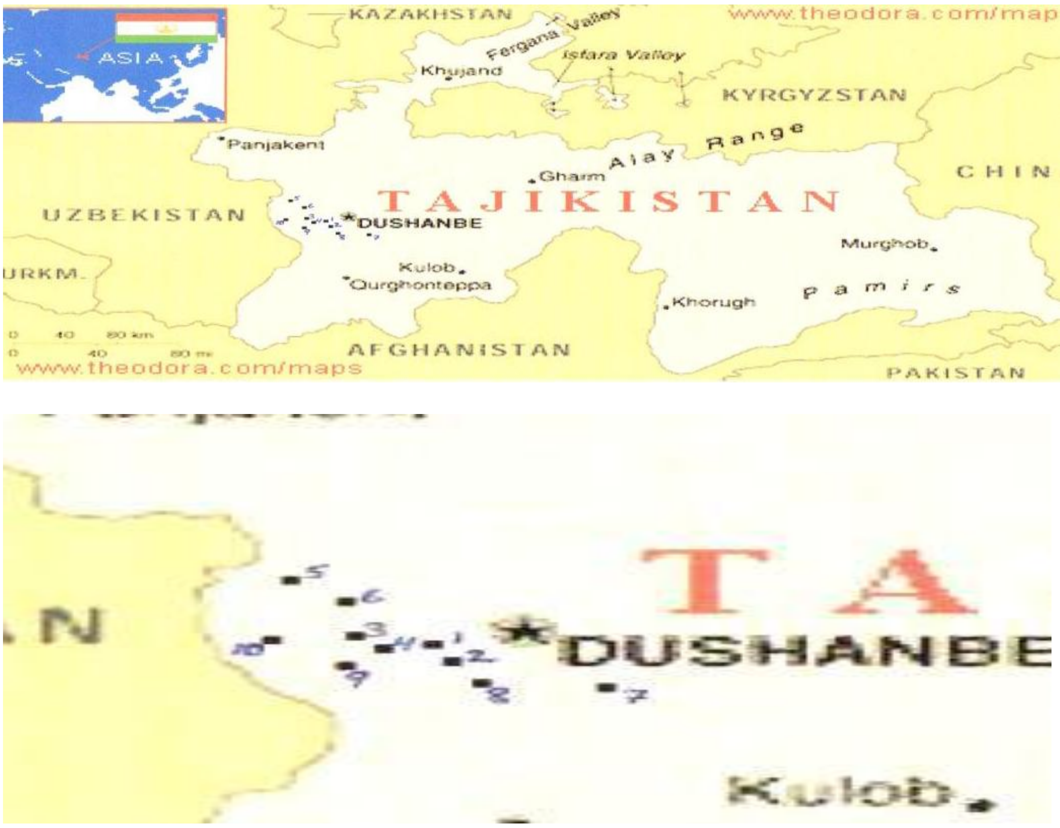
Aim of the study

The aim of this study was to investigate occurrence of *A. marginale* and *B. bigemina* in dairy cattle in western Tajikistan and to study risk factors relevant for controlling these diseases.

MATERIAL OCH METHODS

Study area

The study was conducted in western Tajikistan. The farms included in the study were situated in four different districts close to Dushanbe, the capital of the country. The districts were Rudaki, Shakhrinav, Gissar and Turzunsade (map 2).



Map 2. Map with location of the farms (www.theodora.com/maps)

Most of the farms were situated in the Kofarnihon valley. In this area, the normal yearly precipitation ranges between 250-750 mm (Best Country Reports, 2009). At the time for this study there had been less rainfall than normal and there was water shortage in some farms. The area, including pastures, was very dry (picture 6). Approximately 150.000 cattle were held in the study area (Sattorov, TAU, personal message).

Picture 6. Pasture close to Dushanbe (private photo)

Study population

The selection of herds was made by TAU in cooperation with local veterinarians in the different districts with the aim to include private smallholder and governmental farms. To estimate the prevalence with a 90% confidential interval, 5% precision and an estimated prevalence of 50%, the aim was to sample at least 271 cattle.

The cattle in the study were from private smallholders, state farms and dehkans. In the state farms and dehkans there were between 100 and 900 cattle which were held outside in open pens (picture 7). Many of these farms took the animals to pasture in the summer due to feed shortage. Each private smallholder had approximately 3-4 cattle to provide their own family with milk and meat. The animals were housed during nights and winter close to the owners´ homes (picture 8). During the summer all the private smallholders in a village took their cattle to common pasture. Since the cattle in the villages were spending most of the time during the summer at shared pastures, we considered each village within this study as one epidemiological unit. Every state farm and dehkan was considered as one epidemiological unit.

Picture 7. Cattle of Holstein breed in open pen at a state farm (private photo)

Picture 8. Cattle of Latvian red and Russian black and white breeds in a backyard (private photo)

The cattle included in the study were between 6 months and 15 years old and of Holstein, Russian black and white, local, mixed, Latvian red and Carpat breeds. Holstein and Russian black and white are high producing milking breeds, the local breed is a small breed not producing as much, and the mixed breed are crosses between local breed and the other breeds. Latvian red is a milking breed and Carpat is a beef cow. All cattle were milked by hand twice a day.

Blood sample collection and analysis

Samples were taken from 294 animals selected by personnel and owners of the units. Blood was collected in non-heparinised vacutainers, from the jugular vein or the coccygeal vein. Each sample was labelled with number of epidemiological unit and number of cattle. The samples were stored in a cool-box and transferred to a refrigerator at the laboratory at the TAU. Serum separated spontaneously. The day after collection serum was poured into sterile serumtubes. The tubes were kept in a freezer until time for analysis.

Analysis of the blood samples was made at the Veterinary Research Institute in Dushanbe. Assessment of antibodies against *A. marginale* and *B. bigemina* was conducted using ELISA kits (SVANOVA Biotech AB, Uppsala, Sweden) according to the instructions in the manual. The OD values were expressed as percentage positivity (PP), calculated as follows: (Mean OD value from sample/Mean OD value from positive control) x100. When testing animals for *A. marginale* PP values ≥ 25 were considered positive and PP values <25 were considered negative. When testing animals for *B. bigemina* animals with PP values ≤ 25 were considered negative, animals with PP values ≥ 40 were considered positive and PP values in the grey zone between 26 and 39 were considered inconclusive. According to the manufacturer of the ELISA kits serum samples with PP values that were in the grey zone should be interpreted as inconclusive and assay must be repeated. If the result comes out inconclusive again a new sample should be tested after three weeks. This was not possible to do in this study due to lack of ELISA kits and time.

Tick counts

The density of ticks on individual animals was assessed by counting the ticks on the head, neck and around the base of the tail. Animals with ticks in these areas were also counted for ticks over the rest of the body. Tick counts were recorded in the following categories: 1 for up to 10 ticks, 2 for 11-50 ticks and 3 for over 50 ticks (Magona et al., 2008).

Questionnaire

A questionnaire was brought to every epidemiological unit at the time for blood sampling. The questions were in Russian and answered by the person/persons working at the governmental unit that day or by the owners of the private units.

The answers were translated into English by our Tajik counterpart from the TAU at the time of the visit.

The questionnaire consisted of questions regarding location of the unit, unit structure and type of pastures, age and breed of cattle and animal health, tick burden and use of tick prevention.

Statistic analysis

Data was analyzed using Chi-2 tests to investigate associations between prevalence of the two diseases and the different variables (risk factors). Factors with p≤ 0,05 were considered as statistically significant.

RESULTS

Descriptive results

Blood-samples were collected from 294 cattle. Of these 200 (68%) were taken from animals at the seven state units and dehkans, and 94 (32%) from the three small holder units (figure 1). The animals were of Russian black and white, local, Holstein, mixed, Carpat and Latvian red breeds (figure 2).

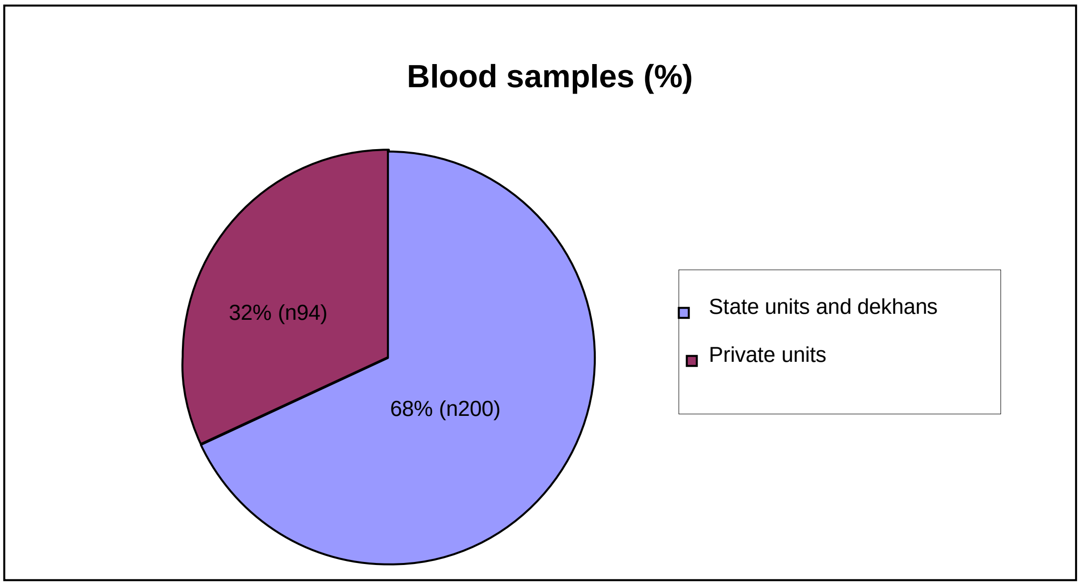


Figure 1. Percentages of blood samples collected from state units, dehkans and private units in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

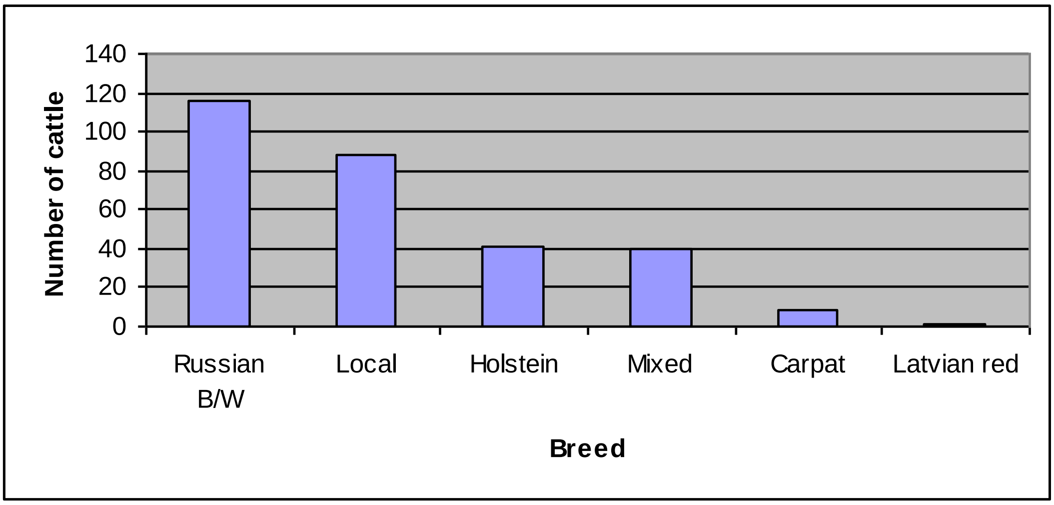


Figure 2. Number of blood samples collected from different cattle breeds in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

Figure 3 shows distribution between age groups. Table 2 shows number of blood samples collected in different regions.

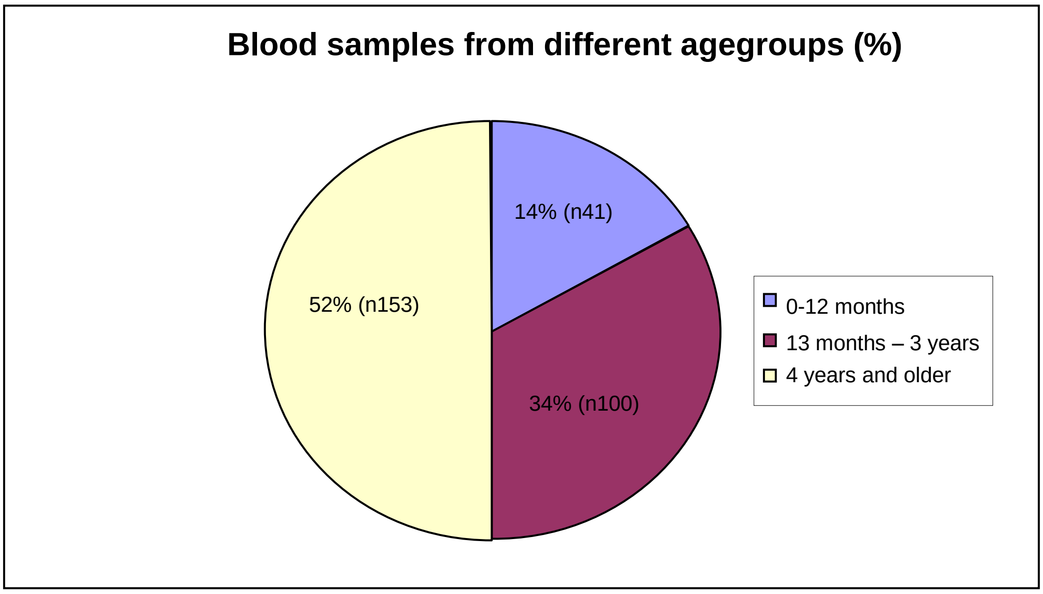


Figure 3. Distribution of blood samples in % collected from different age groups in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

Serological results

Fourty (14%) of the tested animals were *A. marginale* positive (fig 4). Fiftythree (18%) animals were *B. bigemina* positive and 33 (11%) of the samples were considered inconclusive (fig 5). In all sampled units except one there were animals testing positive for *A. marginale* infection and inconclusive and/or positive for *B. bigemina* infection (table 1). Seven (2,5%) of the tested animals were positive both for *A. marginale* infection and *B. bigemina* infection. Another seven (2,5%) of the tested animals were positive for *A. marginale* infection and inconclusive for *B. bigemina* infection (figure 6).

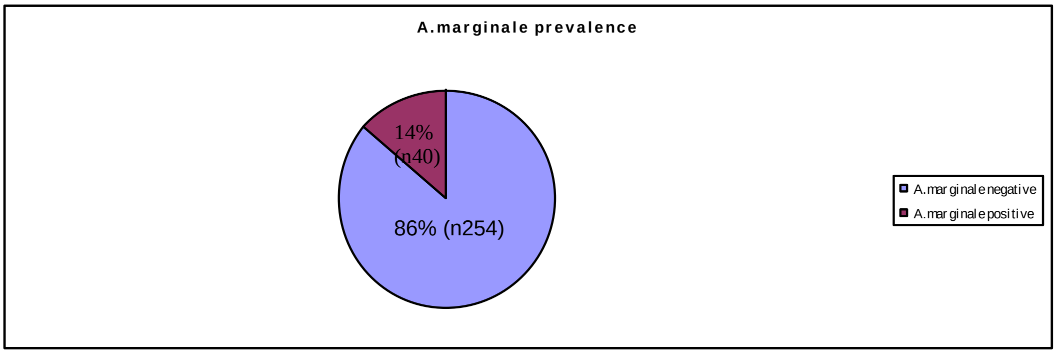


Figure 4. Prevalence of A. marginale seropositive animals in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

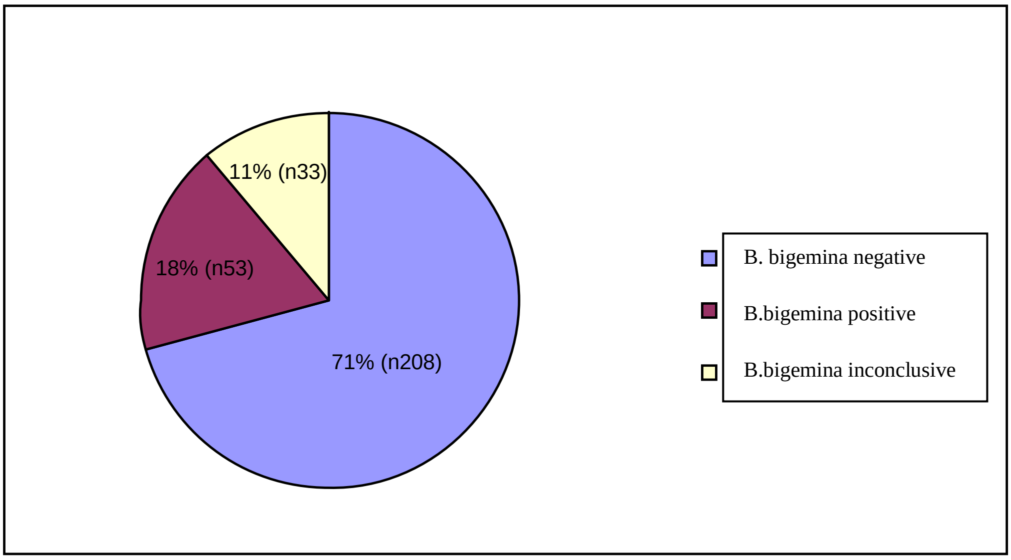


Figure 5. Prevalence of B.bigemina positive and inconclusive results in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

Table 1. Total number of animals, collected samples and number of serological positive and inconclusive testresults of each of the tested infections. in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

Epidemiological unit	Tot.no. animals	Tot.no. samples	Anaplasma No.pos (%)	Babesia No.pos (%)	Babesia No. inconclusive(%)
1	900	10	0 (0,0)	1 (10)	0 (0,0)
2	270	10	2 (20)	2 (20)	0 (0,0)
3	400	35	2 (5,7)	6 (17)	1 (2,9)
4	140	35	2 (5,7)	4 (11)	2 (5,7)
5	18	8	1 (33)	5 (63)	0 (0,0)
6	38	30	8 (27)	6 (20)	9 (30)
7	88	26	4 (15)	2 (7,7)	5 (19)
8	680	34	3 (8,8)	7 (21)	6 (18)
9	420	56	7 (13)	10 (18)	6 (11)
10	384	50	11(22)	10 (20)	4 (8,0)

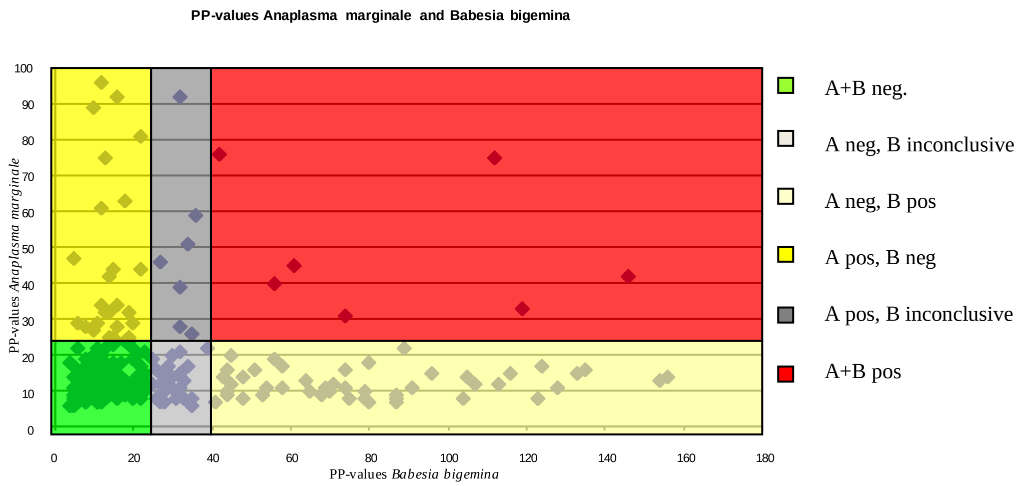


Figure 6. Distribution of pp-values among all the tested animals for infection with A .marginale ('A' in figure) and B. bigemina ('B' in figure) in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

Tick counts

Most of the cattle in the study had less than ten ticks. Only one animal had between 11 and 50 ticks and five animals had more than 50 ticks (table 2). The ticks that were found on the animals were mostly nymph and adult stages of *Boophilus* ticks. Only one tick of *Hyalomma.spp.* was found.

Questionnaire

The results from the questionnaire are summarized in table 2. The answers about tick burden and animal health were difficult to interpret and therefore excluded from the study. Seven epidemiological units took their cattle to pasture and three units did not. Type of tick prevention varied a lot between epidemiological units (table 3).

Statistical results

There was a clear trend of higher prevalence of *Babesia bigemina* positive and inconclusive results in private units than in state and dehkan units (P=0,06). The trend is the same, but not as clear, for *Anaplasma marginale* (P=0,24).

The seroprevalence of *A. marginale* was higher in mixed and local breeds and lower in Holstein cattle (P=0,05). Latvian red and Carpat breeds were excluded because of low numbers of these breeds in the study.

There was a trend of increasing seroprevalence of *A. marginale* with age (P=0,18). There was no such trend for *B. bigemina* were the animals >4 years had the highest seroprevalence, the animals 6-12months had a lower seroprevalence and the animals between 13months - 3years had the lowest seroprevalence.

There was no significant difference in *A. marginale a* and *B. bigemina* seroprevalences between cattle that had tick prophylaxis and cattle that did not.

The number of cattle seropositive for *A. marginale* was higher in the region of Tursunzade, but this was not significant.

Table 2. Seroprevalence of Anaplasma and Babesia infection related to different variables. in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

Variable	Number of samples (%)	Anaplasma No. pos (%)	Babesia No. pos (%)	Babesia No. inconclusive (%)
Unit type				
Private	94 (32)	16 (17)	21 (22)	15 (16)
State/Dehkan	200 (68)	24 (12)	32 (16)	18 (9,0)
Breed				
Russian B/W	116 (39)	11 (10)	23 (20)	13 (11)
Mixed	40 (14)	8 (20)	9 (23)	5 (13)
Local	88 (30)	18 (21)	16 (18)	13 (15)
Carpat	8 (2,7)	0	0	0
Holstein	41 (14)	3 (7,3)	5 (12)	2 (4,9)
Latvian red	1 (0,3)	0	0	0
Age				
6-12 months	41 (14)	3 (7,3)	7 (17)	4 (9,8)
13 months-3y	100 (34)	11 (11)	13 (13)	7 (7,0)
>4 years	153 (52)	26 (26)	33 (22)	22 (14)
Pasture				
Yes	284 (84)	34 (14)	48 (19)	28 (11)
No	46 (16)	6 (13)	5 (10)	5 (11)
Tick prophylaxis				
Yes	230 (78)	32 (14)	38 (16)	27 (11)
No	64 (22)	8 (13)	15 (23)	6 (9,4)
No. of ticks				
0-10	288 (98)	39 (13)	49 (17)	33 (11)
11-50	1 (0,3)	0 (0,0)	1(100)	0 (0,0)
>51	5 (1,7)	1 (20)	3 (60)	0 (0,0)
Region				
Gissar	54 (18)	5 (9,3)	10 (19)	6 (11)
Rudaki	26 (8,8)	4 (15)	2 (7,7)	5 (19)
Shakhrinav	134 (46)	12 (9,0)	25 (19)	9 (6,7)
Tursunzade	80 (27)	19 (24)	16 (20)	13 (16)

Table 3. Seroprevalence of anaplasma and babesia infection related to tick prophylaxis in a study of anaplasmosis and babesiosis in western Tajikistan 2008.

Tick prophylaxis	Anaplasma No. pos (%)	Babesia No. pos (%)	Babesia No. unsure (%)	Total No. of samples
Racidol				
4 times per month	2 (10)	3 (15)	0	20
3 times per month	15(22)	13(19)	9(13)	67
2 times per month	4 (5,7)	10 (14)	3 (4,3)	70
1 times per month	3 (33)	3 (33)	4 (44)	9
Sebasil				
2 times per month	3 (8,8)	7 (21)	6 (18)	34
3 times per month	4 (15)	2 (7,7)	5 (19)	26
Niazid+Dodst				
2 times per summer	1 (25)	0 (0,0)	0 (0,0)	4

DISCUSSION

In an area of endemic stability the seroprevalence is over 60% and clinical disease is rare (Radostits et al, 2007). On the other hand, in areas of endemic instability with lower seroprevalence clinical disease is common and often fatal. As much as 18% of all cattle in an area of endemic instability show clinical symptoms of babesiosis (Taylor et al, 2007). Based on our results and the definition above it may be concluded that an unstable endemic state for anaplasmosis and babesiosis probably exists in the study area. Therefore one can assume that these diseases cause clinical illness followed by production- and income losses. The economical impact of TBDs is hard to define because of its complexity, and therefore often neglected. According to ruff estimates based on Australian figures (14 US dollars per cattle) assuming that 80% of all cattle are at risk of infection, the economical loss in the whole world is as much as 18 billion US dollars (de Castro, 1997). Transferred to Tajikistan this would mean economical losses of around 15 million US dollars estimated on 1,1 million cattle in the country. These figures are only estimates but indicate that TBDs cause large economical losses and in a poor country like Tajikistan where so many people are dependent on healthy livestock, TBDs can have a major negative impact both on families welfare and national economics, and should therefore be taken seriously.

During my visit in Tajikistan theileriosis was often referred to as the most important TBD. In my study the seroprevalence of *A. marginale* was 14% and of *B. bigemina* 18%. In another study made in Tajikistan the seroprevalence of *A. marginale* was 18% (Sakhimov, 1997). These results implicate that these infectious agents should be taken in consideration when working against TBDs in Tajikistan. In one study in Uzbekistan the prevalence of *B. bigemina* was 23% (Ilham Rasulov, 2007). Because of a different study population and method of analyzing samples this figure is not possible to compare to results from this study. It would be very interesting to compare seroprevalence of *T. annulata* whith seroprevalence of *A. marginale* and *B. bigemina* using comparable analytic methods.

Diagnosis in the acute phase of TBD infection in Tajikistan is often depending on clinical signs (personal message, TAU). Since the clinical signs are similar for anaplasmosis, babesiosis and theileriosis one can not exclude the risk of making wrong diagnosis. There are methods of analyzing which disease is causing acute clinical signs in cattle, for example Giemsa staining of blood smears (used in Tajikistan) and PCR. The ELISA test used in my study measures seroprevalence but not clinical disease or how severe the disease is. It would therefore be very interesting to investigate which of these diseases cause most clinical signs of TBDs among cattle in Tajikistan.

In Uzbekistan ticks of *Boophilus spp.* and *H. anatolicum* appear during spring, summer and autumn (Rasulov, 2007). Since Uzbekistan and Tajikistan have similar climate it is probable that these ticks have the same seasonal distribution in Tajikistan. In this study there were not many ticks found on the animals. This could be a consequence of the low amount of rainfall and the time of the tick count. Maybe more ticks would have been found if they were counted at a time more favourable for ticks, for example after rain in May or June.

Antibody titers are highest at the time of the active phase of anaplasmosis (Radostits et al, 2007). Both in *A. marginale* and *B. bigemina* infections the causative agent is most active in one to two weeks after infection. After the acute phase the antibody titers become gradually lower in a couple of months but remain for a varying time depending on if the animal is reinfected or not. However, considering that antibody titers are high for some time after infection and that highest tick infestation (and infection) is most likely during the time prior to when this study was conducted, it was a favourable period to investigate seroprevalence with ELISA technique.

There was no difference in *A. marginale* or *B. bigemina* seroprevalence between the group that used tick prophylaxis and the group that did not. It is hard to make any conclusions out of this because the wide range of different prophylaxis and different interval for application of prophylaxis used in the study area. It is also uncertain if the information about prophylaxis is reliable.

According to previous studies (Radostits et al., 2007) *B. indicus* cattle are more resistant to tick infestation and TBD infections than *B. taurus* cattle. Despite this fact, in this study, Holstein breed had a significant lower seroprevalence than local and mixed breeds. This is probably due to high number of Holstein breed in governmental units were *A. marginale* and *B. bigemina* seroprevalence was lower than in private units where only one cattle of Holstein breed was sampled. *A. marginale* and *B. bigemina* seroprevalence was higher in private units where a lot of cattle of local breed were held. One reason for the lower seroprevalence at governmental units could be that there was a more systematic use of tick prophylaxis and that animals at these units were kept at pasture to a lesser extent compared with private units. In Tajikistan 80% of the cattle are held at private units. Since so many people are dependent on these animals to be healthy it would be helpful to define the reason why *A. marginale* and *B. bigemina* seroprevalence is higher in private units. None of the variables compared in this study (unit type, breed, age, pasture, prophylaxis, number of ticks and region) except breed, came out to be a significant risk factor for *A. marginale* or *B. bigemina* seroprevalence. This might be due to the great number of units included in this study with different external variables that influence *A. marginale* or *B. bigemina* seroprevalence. To compare risk factors it would probably be helpful to limit the number of units and extend the number of sampled animals from each unit.

Almost one third of the study population had serum antibodies against one of the two diseases included in this study. Seven animals were infected with both both *A. marginale* and *B. bigemina* and all but one were four years or older. Animals in this age category had the highest amount of seropositive animals. It is hard to make any conclusions out of this, because amount of antibodies and how long they last varies a lot between individuals.

CONCLUSION

Infections caused by *A. marginale* and *B. bigemina* are present among dairy cattle in western Tajikistan.

Western Tajikistan is, based on results from this study and previous knowledge of these diseases, a non-endemic area where *A. marginale* and *B. bigemina* infections probably cause clinical illness resulting in losses in milk and meet production.

Further studies are needed to appoint to what extent clinical disease is present, to increase knowledge about factors affecting prevalence, and to estimate how great the impact on production is following *A. marginale* and *B. bigemina* infections in cattle in western Tajikistan.

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